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## Thrombus NET content is associated with clinical outcome in stroke and myocardial infarction

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**Abstract:** **OBJECTIVE** To investigate whether immune cell composition and content of neutrophil extra-cellular traps (NETs) in relation to clinical outcome are different between acute ischemic stroke (AIS) and acute myocardial infarction (AMI), we performed histologic analysis and correlated results with clinical and procedural parameters. **METHODS** We retrieved thrombi from patients with AIS (n = 71) and AMI (n = 72) during endovascular arterial recanalization and analyzed their immune cell composition and NET content by immunohistology. We then associated thrombus composition with procedural parameters and outcome in AIS and with cardiac function in patients with AMI. Furthermore, we compared AIS thrombi with AMI thrombi and differentiated Trial of Org 10172 in Acute Stroke Treatment classifications to address potential differences in thrombus pathogenesis. **RESULTS** Amounts of leukocytes (p = 0.133) and neutrophils (p = 0.56) were similar between AIS and AMI thrombi. Monocytes (p = 0.0052), eosinophils (p < 0.0001), B cells (p < 0.0001), and T cells (p < 0.0001) were more abundant in stroke compared with AMI thrombi. NETs were present in 100% of patients with AIS and 20.8% of patients with AMI. Their abundance in thrombi was associated with poor outcome scores in patients with AIS and with reduced ejection fraction in patients with AMI. **CONCLUSION** In our detailed histologic analysis of arterial thrombi, thrombus composition and especially abundance of leukocyte subsets differed between patients with AIS and AMI. The presence and amount of NETs were associated with patients' outcome after AIS and AMI, supporting a critical impact of NETs on thrombus stability in both conditions.

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# A deep learning oriented method for automated 3D reconstruction of carotid arterial trees from MR imaging

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**Abstract**— The scope of this paper is to present a new carotid vessel segmentation algorithm implementing the U-net based convolutional neural network architecture. With carotid atherosclerosis being the major cause of stroke in Europe, new methods that can provide more accurate image segmentation of the carotid arterial tree and plaque tissue can help improve early diagnosis, prevention and treatment of carotid disease. Herein, we present a novel methodology combining the U-net model and morphological active contours in an iterative framework that accurately segments the carotid lumen and outer wall. The method automatically produces a 3D meshed model of the carotid bifurcation and smaller branches, using multispectral MR image series obtained from two clinical centres of the TAXINOMISIS study. As indicated by a validation study, the algorithm succeeds high accuracy (99.1% for lumen area and 92.6% for the perimeter) for lumen segmentation. The proposed algorithm will be used in the TAXINOMISIS study to obtain more accurate 3D vessel models for improved computational fluid dynamics simulations and the development of models of atherosclerotic plaque progression.

## I. INTRODUCTION

Carotid atherosclerosis is considered the major cause of ischaemic cerebrovascular events in Europe, increasing morbidity and mortality rates and imposing high

socioeconomic burden to patients, family members and healthcare systems. Carotid atherosclerotic plaques lead to progressive luminal stenosis of the vessel, which may erode or rupture causing cerebral infarction and stroke [1].

Magnetic Resonance Angiography (MRA) is an imaging modality widely used for the diagnosis and management of carotid artery disease. High resolution images provided by MRA help clinicians detect atherosclerotic plaques even at early stages, evaluate their stability and support clinical decision making. In this context, numerous studies have demonstrated that there is a link between the biomechanical forces applied in the vessel by blood flow, and the development and progression of atherosclerotic plaque tissue [2, 3]. MRA images can be used for patient specific 3D reconstruction of the vessel's lumen and outer wall, and after applying computational fluid dynamics (CFD) simulations, the calculated biomechanical forces can be associated with plaque tissue characteristics and cerebrovascular events such as stroke [2, 3]. For this reason, new imaging methods and techniques that can enhance visualization of the vasculature and improve image segmentation of the lumen, wall and plaque tissue can provide more accurate 3D vessel models and valuable information for the better diagnosis, prevention and treatment of carotid atherosclerotic disease.

Herein, we present the implementation of a U-net based convolution neural network algorithm applied for the image segmentation of the carotid arterial tree. Images were provided by multi-spectral magnetic resonance imaging (MRI) series obtained from patients enrolled in two clinical centres in the TAXINOMISIS study. The U-net architecture is implemented in an iterative framework combining morphological active contours to successfully and automatically produce a 3D reconstructed carotid artery tree including the bifurcation and upstream branches. The overall methodology and preliminary validation results are presented.

## II. MATERIALS AND METHODS

### A. MRI 3D Reconstruction Methodology

Accurate and automated 3D geometrical reconstruction of carotid arterial trees is vital for the understanding of the pathogenesis of atherosclerosis. Within this section, a novel methodology for reconstructing carotid arterial trees in an automated and real-time fashion using as input multispectral MRI series is presented. The proposed methodology utilizes texture based image processing algorithms and machine

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learning models in a hybrid iterative framework and can accurately provide 3D meshed models of the lumen and the outer arterial wall, as evidenced by the validation study reported in section III.

of-Flight (TOF) and the T1-weighted (T1W) image series, the two series (TOF and T1W) are registered by assigning for each T1W the “closest” TOF frame.

MRI Carotid artery Segmentation methods	Image Processing				Machine Learning	Hybrid Models	Description	
	Deformable models			Graph-based models				
	Active Contour		Level Set		Graph Cut			
	2D	3D	2D	3D				
Adame IM, et al., 2004 [4]	ellipse fitting				Fuzzy c-means	Hybrid model	Automatic, ellipse fitting for vessel wall boundary and fuzzy clustering for lumen and carotid plaques.	
Van't Klooster R. et al., 2012 [5]		3D NURBS surface		Fast Marching LS			Automatic 3D cylindrical NURBS surface using wavefront propagation based on Fast Marching Level Sets	
Tang H., et al., 2012 [6]				Geodesic LS			Semiautomatic 3D geodesic level set active contour	
Hameeteman K, et al., 2013 [7]		3D NURBS curves			AdaBoost	Hybrid model	Automatic 3D NURBS plus AdaBoost classifier and kernel radius for post processing corrections	
Saba L., et al., 2014 [8]	2D GVF		2D LS				Semiautomatic LS vs GVF detection of lumen and wall boundaries	
Jodas DS, et al., 2016 [9]	Chan-Vese AC				K-means	Hybrid model	Automatic K-means clustering for lumen segmentation & Chan-Vese AC refinement	
Arias-Lorza AM, et al., 2016 [10]					3D Optimal Surface Graph Cut		Semiautomatic 3D Optimal Surface Graph Cut for Carotid bifurcation	
Gao S., et al., 2017 [11]		3D model fitting				K-NN	Hybrid model	Automatic hybrid 3D deformable model fitting with KNN boundary classification. Hierarchical-tree model used for the bifurcation
Arias-Lorza AM, 2018 [12]					3D Optimal Surface Graph Cut	SVM	Hybrid model	Automatic 3D Optimal Surface Graph Cut using Regional Probability Maps computed by SVM

**Table 1.** State-of-the-art methods for carotid lumen and outer wall segmentation based on multispectral MRI sequences.

### A1. Overall approach

Focusing on the research work in the field during the last five years, one can conclude that the main approaches for image segmentation mainly involve active contours (with various objective functions, like snakes or level sets) both in 2D and in 3D domains and machine learning (ML) approaches, which are produced by training different models (NNs, SVMs, etc.) with annotated data (Table 1). Moving one step further, the proposed methodology contributes to the state-of-the-art approaches by:

1. Training and deploying a deep learning model (U-net) for the image segmentation phase and
2. Utilizing an iterative approach, according to which the produced model is refined over several iterations, aiming to reach certain quality criteria, based on the anatomic properties of the specific body area.

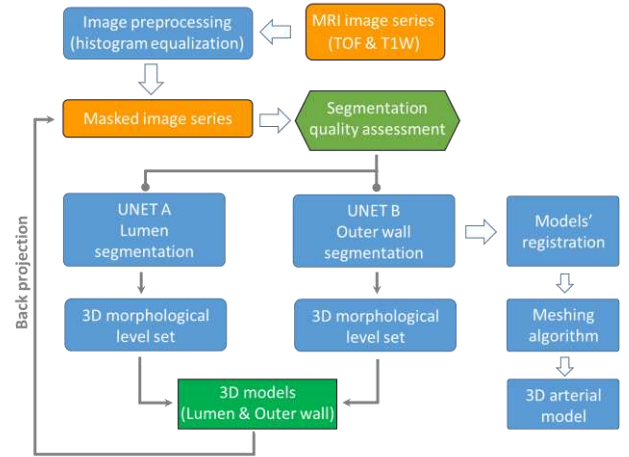
The overall methodology is presented in Fig. 1.

### A2. Image preprocessing algorithms

Preprocessing is crucial for the proposed methodology, as it provides the capacity to the described algorithms to use MRI sequences from various image acquisition setups. The first step of the preprocessing is the automated isolation of the image region which contains the arterial tree under reconstruction. This is performed by selecting a static 100x100 pixels window (either for the left or for the right arterial model). The coordinates of this window reassure that the whole arterial tree is included in the cropped region. On the cropped region, an adaptive histogram equalization filter is applied, aiming to generalize the input sequence for the next steps. Finally, based on the DICOM tags of the Time-

### A3. Image segmentation model

One of the most important components of the methodology described in the previous section is the segmentation component, which is used to isolate the pixels of interest from each MRI series (lumen from the TOF series and outer



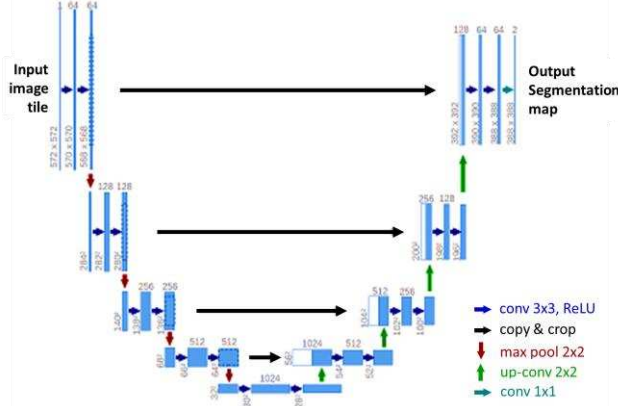
**Figure 1.** Schematic presentation of the proposed methodology for the segmentation and 3D reconstruction of the carotid arterial tree.

arterial wall from the T1W series). For this, the U-net model has been utilized [13], as it has shown remarkable results on medical image segmentation tasks (Fig. 2).

#### A3.1. Training dataset

In order to train the U-net model, a set of MRI examinations has been collected. MRI examination was performed in 30 patients on a 1.5-T whole-body system (Signa HDx, GE Healthcare, Waukesha, WI, USA) using a bilateral four-channel phased-array carotid coil (Machnet

BV, Eelde, the Netherlands). All patients gave informed written consent, and the study protocol was approved by the local regional ethics committee. The acquisition parameters for the MRI sequences were as follows: (i) TOF images: repetition time: 23 ms, effective echo time: 3.2 ms, field of view [FOV]: 160 mm, section thickness: 1 mm; (ii) fast-spin echo double-inversion recovery prepared sequences (T1W):

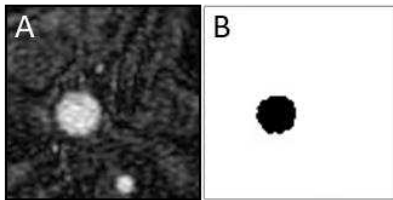


**Figure. 2.** U-net architecture (example for 32x32 pixels in the lowest resolution). Each blue box corresponds to a multi-channel feature map. The number of channels is denoted on top of the box [13].

repetition time: 1428.57 ms, effective echo time: 7.672 ms, FOV: 100 mm, section thickness: 3 mm; The acquired data were stored in DICOM format.

#### A3.2. Training dataset preparation

Two independent specialists annotated the luminal wall on the TOF frames and the outer arterial wall on the T1W frames. Based on these annotations, masked images have been created, which are used as input for the training phase of the U-net models (one model for segmenting the lumen and one model for segmenting the outer arterial wall), as depicted in Fig. 3.



**Figure. 3.** (A) TOF image and (B) the corresponding mask image used for U-net training.

This process resulted in two datasets, one for the luminal wall with 30 (patients) x 100 (TOF frames) x 100 x 100 pixels and one for the outer arterial wall with 30 (patients) x 10 (T1W frames) x 100 x 100 pixels. This deep neural network was implemented with PyTorch functional API. Output from the network is a 100 x 100 pixels image which represents the segmented image. In order to reassure that the activation function was utilized, while the binary cross-entropy loss function was selected.

#### A4. 3D level set segmentation

The segmented images derived by the deep neural network are stacked and create a pixel volume (one for TOF and one

for T1W). On the created volumes, a 3D level set is applied, which used as kernel the curvature morphological operator proposed by [14]. Eq. (1) summarizes the utilized morphological operator.

$$E(s) = \iint g(I)(S(a)) da, \quad (1)$$

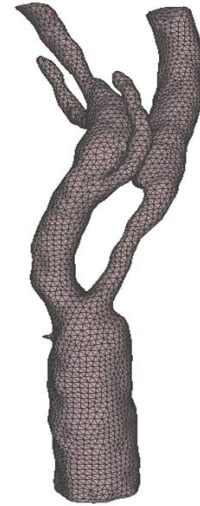
where  $da$  is the Euclidean element of area, and  $g(I)$  allows the isolation of the region of interest. The 3D level set model is initialized as a cylinder around the volume and gradually deforms on the arterial tree. This procedure results to a cloud point of the segmented pixels.

#### A5. Iterative process

The cloud point of the previous procedure is back projected on the initial frames. Then, a connected components calculation algorithm is applied, aiming to detect on each frame all the remaining contours. Based on the anatomical features of the specific area, one would expect to have one object before the bifurcation, two objects just after the bifurcation and three to four objects 10mm above. When these criteria aren't met, the algorithm masks the frames based on the latest received level set and feeds models A and B again, aiming to refine the segmentation. This process continues until an anatomically valid model is produced, or after ten iterations.

### III. RESULTS

Fig. 4 illustrates a carotid arterial tree reconstructed using the proposed methodology.



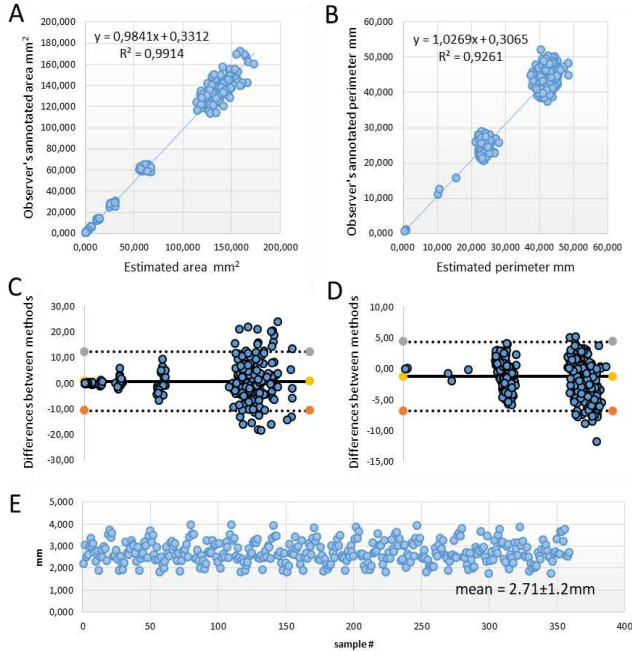
**Figure. 4.** 3D reconstructed arterial tree with a significant degree of luminal stenosis in the internal carotid artery, upstream the bifurcation site.

A validation study on the luminal arterial wall has been performed, in order to assess the accuracy of the proposed scheme. For this, ~200 TOF images were annotated by specialists. On each frame, the specialist's annotation was treated as the ground truth and the lumen area, the lumen perimeter and the Hausdorff distance were calculated. Fig. 5 summarizes the validation study.



#### IV. DISCUSSION

MRA is widely used in the diagnosis of vascular diseases such as carotid atherosclerosis. Multispectral MRA images can be used for patient specific 3D reconstruction of the carotid arteries and allow the detection and evaluation of atherosclerotic plaques and their stability, a strong predictor



**Figure 5.** Validation study with Linear Regression analysis of the lumen area (A) and perimeter (B), Bland Altman Analysis of areas (C) and perimeters (D), and Hausdorff Distance (E) between automatic and manual segmentation.

of cerebrovascular events such as stroke. While manual segmentation of the carotid artery and its components has been used by experts, it is an expensive task in terms of time and effort and also linked with intra- and inter-observer variability. Semi-automatic and automatic approaches overcome these hurdles but still require proper annotation by expert clinicians to evaluate the results obtained by segmentation algorithms. To this end, and concerning the segmentation of carotid arteries based on MRI multispectral series, researchers have used image processing approaches, machine learning approaches and hybrid models (Table 1) to segment the lumen and outer wall of the vessel. In this paper, we presented a novel carotid artery segmentation algorithm that utilizes texture based image processing algorithms and a U-net deep learning model in a hybrid iterative framework that can accurately and automatically provide 3D models of the carotid bifurcation and smaller branches. Although, the proposed algorithm has yet to be fully validated in terms of larger datasets during the TAXINOMISIS study, the preliminary validation results presented here are quite promising.

#### V. CONCLUSION

With medical imaging technology and methods constantly advancing, the proposed hybrid methodology is a promising new approach to produce more accurate 3D reconstructed

models of the carotid artery and potentially plaque tissue components that can help improve early diagnosis, prevention and treatment of carotid artery disease.

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